THE EFFECTS OF CARBON DIOXIDE ON THE CONSISTENCY OF PROTOPLASM.

M. H. JACOBS,

UNIVERSITY OF PENNSYLVANIA.

INTRODUCTION.

It has been shown by a number of recent investigators, using the microdissection and other methods, that the consistency of the protoplasm of different cells may vary from that of a liquid almost as mobile as water to that of a solid gel. It has also been shown that within the same cell at different times there may be almost equally striking differences. For example, Bayliss ('20) has reported that when the ectoplasm of an *Amœba* is observed with the ultramicroscope it appears to be filled with fine brilliant particles in rapid Brownian movement—an indication of the liquid state. On electrical stimulation the movement suddenly ceases, to be resumed when the stimulation is discontinued. Evidently in this case stimulation is attended by a solidification, or at least by a very considerable increase in the viscosity of the protoplasm. Another important example is furnished by cells in the act of division. Heilbrunn ('15) and Chambers ('17), using entirely different methods, have both shown that during the cleavage of marine ova there is with each cell-division a regular cycle of changes leading from a more liquid to a more solid condition and back again. It seems very likely that these changes play an important part in the actual mechanics of the division process. A third example is furnished by various cases of amœboid movement, where changes in protoplasmic consistency are thought by some of the most recent workers on the subject (especially L. Loeb, '02, '20, '21, etc.) to be concerned in actually producing the movement. A considerable number of other cases, some definitely established, others merely probable, could be mentioned. It seems certain that changes in protoplasmic consistency are not only of widespread occurrence, but are also of great physiological importance.

As to the cause of these changes, little is known. Heilbronn ('14) and Heilbrunn ('20) have shown that such effects can be produced by changes in temperature, but evidently this factor is not an important one in the cases that have been mentioned. Szücs ('13) showed that aluminium salts could bring about a solidification followed later by a liquefaction (both reversible) in plant cells, while Heilbronn, and especially Heilbrunn, have obtained striking results with ether, chloroform and other anæsthetics. Important as are these various studies on the effects of chemicals in throwing light on the nature of protoplasm, they give little information as to the cause of the physiological changes in viscosity observed under natural conditions, since the substances employed have not been such as are usually encountered in living cells.

In the course of some work done on a different problem several years ago the author observed certain effects of carbon dioxide on the protoplasm of living Protozoa which suggested the desirability of testing to what extent this substance might be able to cause reversible changes in protoplasmic viscosity. If it could be shown that it does, in fact, possess such powers in any striking degree, the observation would be a suggestive one, since CO_2 is not only a universal product of cell activity, but it has been definitely shown in certain cases (Lyon, '04) to be produced at different rates at different times within the same cell, and a plausible hypothesis might in that event connect the observed changes in viscosity, in part at least, with varying rates of CO_2 production. In the present paper evidence is presented that changes of the sort demanded by such an hypothesis may, as a matter of fact, be produced.

The material used consisted of the two Protozoa, *Paramœcium caudatum* and *Colpidium colpoda*, the eggs of the sea-urchin, *Arbacia*, and in a few experiments filaments of *Spirogyra*. In addition, a number of observations were made on *Amœba*. The consistency of the protoplasm of all of the cells except *Amœba* was tested by the centrifuge method of Szücs and of Heilbrunn, advantage being taken of the fact that the more viscous the protoplasm is, the more difficult it is to bring about a separation from it by means of centrifugal force of solid bodies of different specific gravity contained in it. The cells studied either contained bodies

capable of responding to this treatment (pigment and other granules and droplets of *Arbacia* eggs, chloroplasts of *Spirogyra,* certain fine granules in *Colpidium*) or such bodies could be introduced in the form of food vacuoles filled with India ink (*Paramœcium* and *Colpidium*). The force used with the *Arbacia* eggs was estimated at 1,600 times and with the other material at approximately 150 times that of gravity. The *Arbacia* eggs were centrifuged in sea water, *Spirogyra* in pond or in distilled water, and the Protozoa for the most part in their own culture fluid.

A portion of the experiments here described were carried out at the Marine Biological Laboratory at Woods Hole. The author wishes to acknowledge his indebtedness to the Director, Professor Frank R. Lillie, for placing at his disposal the facilities of the laboratory during a part of the summer of 1920.

Experiments on Paramœcium.

The procedure most frequently employed with this form was as follows: The animals were first placed in a test tube with about 20 c.c. of culture fluid to which a few drops of a dense suspension of India ink had been added. They were then allowed to stand for 30 minutes, at the end of which time they contained numerous black food vacuoles whose behavior could be followed with the greatest ease. Before exposure to carbon dioxide they were tested to determine the ease with which the food vacuoles could be separated from the remainder of the protoplasm. It was found in all the experiments with the culture used that a two minutes' exposure to a force of 150 times gravity caused a partial separation—*i.e.,* there was a general tendency for the ink to move toward the posterior end of the body—but it did not by any means become massed there, and in some individuals no movement at all was evident. When, at the end of 30 minutes, the animals had formed sufficient food vacuoles, a current of carbon dioxide was allowed to bubble through the liquid containing them, and samples were removed at intervals, immediately centrifuged for the same time and at the same rate as before, and then examined in Syracuse watch glasses. After this examination the watch glasses were in each case exposed to the air to allow the escape of the CO_2, and

after several hours another examination was made to determine whether or not deaths had occurred. A considerable number of experiments were made on *Paramœcium*, and as they all showed a very satisfactory agreement in their general results, it will be sufficient to describe in detail only the following typical one.

The animals used in this experiment responded to centrifugalization before treatment with CO_2 in the usual manner—*i.e.*, by a partial, but by no means complete, separation of the food vacuoles from the protoplasm when the force employed and the time of exposure to it were as already mentioned. After the current of CO_2 had flowed for one minute a slight increase in the readiness with which separation occurred was noted; this had decidedly increased by the end of 5 minutes, and by the end of 10 minutes it had become very striking. The India ink after centrifugalization was now invariably found in a compact mass in the posterior end of the body, so closely packed that the outlines of the individual food vacuoles could scarcely be distinguished. The animals themselves at this stage of the experiment showed no visible injury either from the exposure to the carbon dioxide or to the centrifugal force. When placed in open watch glasses they not only continued their locomotion in a normal fashion, but the food vacuoles within a few minutes had become redistributed throughout the cell and the animals had resumed their original appearance.

After 15 and 20 minutes, respectively, from the beginning of the experiment the results were essentially the same as those at the end of 10 minutes, except that a swelling of the body was now becoming increasingly apparent. At the end of 25 minutes a new appearance could be noted. In a few of the individuals, although the treatment was the same as before, the food vacuoles failed to separate. The numbers of such animals gradually increased during the next 15 minutes, until at the end of 40 minutes it was the exception rather than the rule for such separation to occur, and 5 minutes later only a few showed any movements of the food vacuoles at all. At 50 minutes this condition had become practically complete, and the protoplasm of the animals was now evidently considerably more viscous than at the beginning of the experiment. This increased viscosity—or perhaps solidification—

was also made apparent by the cessation of all movements of the contractile vacuoles. Swimming, however, continued, though at a slower rate than before.

All of the effects so far described were found to be completely reversible. Even after the protoplasm had passed through its period of liquefaction and had become much more solid than at the beginning of the experiment the animals practically all recovered normally when the CO_2 was allowed to escape. From 56 minutes onward, however, evidences of fatal injury began to appear. The animals exposed for this length of time showed a mortality of about 25 per cent., though those that did not die recovered entirely normally. By the end of 65 minutes the mortality had increased to approximately 75 per cent., and 10 minutes later to 100 per cent. Since solidification began to be evident in some individuals in 25 minutes and was present in nearly all in 40 minutes, and since scarcely any deaths occurred before 50 minutes, it is evident that there is a considerable period within which the solidification is completely reversible. With an exposure of longer duration, however, it apparently merges imperceptibly into an irreversible death coagulation.

The effects so far described are much more rapidly produced if the animals have been kept in distilled water for a time before their exposure to carbon dioxide. For example, in one experiment a certain degree of liquefaction was produced in animals in the normal culture medium in 12 minutes; in individuals from the same culture which had previously been washed in distilled water this point was reached in less than 6 minutes. The times for the beginning of solidification in the two cases were 40 and 12 minutes, respectively, and while the animals in the culture medium were still living at the end of 60 minutes, those in the distilled water were dead in 32. These differences are perhaps partly due to the greater hydrogen-ion concentration of the distilled water, but experiments that need not be described here have shown that this is not the only factor concerned. The situation is, in fact, a complex one, presenting a number of points of interest, and its fuller discussion in another paper is contemplated.

At this point it may perhaps be well to consider two questions

that might justly be raised in connection with the facts that have been mentioned. The first is whether the behavior of *Paramœcium* may not be due to the removal of oxygen by the current of CO_2 rather than to the action of the latter substance itself; the second is whether the changes in the readiness of separation of the food vacuoles from the protoplasm may not be due to changes in the specific gravity of the latter brought about by the absorption or the giving off of water rather than to mere changes in viscosity.

As to the first question, it may be said that a vigorous current of hydrogen, which must remove the oxygen at least as effectively as the slower current of CO_2, was not found to bring about the sequence of changes described, even when allowed to flow for a considerably longer time. It did, to be sure, appear after a time to favor liquefaction of the protoplasm, but not only was its action in this respect far slower than that of carbon dioxide, but in no case were evidences of subsequent solidification observed though the experiments were continued much beyond the time required for the solidifying action of CO_2. As to the second of the two questions, it may be noted that there appears to be no evident correlation (as there should be if the chief factor were a change in the specific gravity of the protoplasm) between the degree of swelling in *Paramœcium,* which is evidently due to the taking up of water, and the ease with which the food vacuoles may be separated from the protoplasm. A second and even more convincing argument in favor of the view here adopted will be given when the experiments on *Colpidium* are described.

Experiments on Colpidium.

The behavior of *Colpidium* when exposed to carbon dioxide agrees in its essential features with that already described for *Paramœcium*. There is a preliminary period of liquefaction followed by increasing reversible solidification, and finally an irreversible coagulation. *Colpidium* is, however, more resistant to CO_2 than *Paramœcium* and the time required to bring about the final coagulation is considerably greater. This difference is especially striking when the animals have first been placed in distilled water—in fact, it is so great in that case that it may be put to

practical use in converting a mixed culture of *Paramœcium* and *Colpidium* into a pure culture of *Colpidium*. In case it is desired to make such a separation, it is only necessary to wash the animals in distilled water, concentrate them by gentle centrifugalization, and then introduce them suddenly into distilled water one half saturated with carbon dioxide. At the end of a few minutes *Paramœcium* will be found to be dead and *Colpidium* living; the CO_2 may then be removed from the water, a little dry hay added, and an excellent pure culture of the latter organism will be obtained.

Another minor difference between *Colpidium* and *Paramœcium* is that the former in its natural state appears to offer greater resistance to the separation of the food vacuoles by centrifugal force. While an exposure of two minutes, in the culture used, brought about a very distinct separation in *Paramœcium,* one of four minutes was only slightly effective in the case of *Colpidium*. One result of this greater difficulty in bringing about separation in the normal animals is that while it is very easy in *Colpidium* to demonstrate the preliminary liquefaction followed by an increasing solidification which finally leads to a viscosity at least as great as that at the start of the experiment, it is not possible to show conclusively, as in the case of *Paramœcium,* that this viscosity produced by CO_2 is actually greater than that in the untreated animals.

A third point of difference between the two forms is that in *Colpidium*—at least in the individuals studied—there are present, in addition to the food vacuoles, many fine dark particles scattered irregularly through the protoplasm. The effect of centrifugal force after liquefaction has occurred is to throw these particles to the anterior end of the body, while the food vacuoles containing ink go to the posterior end, giving a very distinct banded appearance to the animals, the anterior band being gray, the middle one transparent and the posterior one black. The appearance is very striking and can be seen even with a hand lens. With the centrifugal force used it was never obtained except in animals that had been exposed a moderate length of time to carbon dioxide. In individuals not exposed at all, or exposed a longer time, the banding was indistinct or entirely lacking.

This characteristic of *Colpidium* is of some importance in connection with the question already raised as to whether the differences in the ease of separation of the food vacuoles after exposure to CO_2 are due to changes in the viscosity or in the specific gravity of the protoplasm. In the case of the fine particles and the food vacuoles of *Colpidium* we are evidently dealing with two materials of different densities—one heavier and the other lighter than the protoplasm. If a change in the specific gravity of the latter through the taking up or giving off of water were alone concerned in facilitating separation, it is evident that a change that would favor the movement of the food vacuoles would hinder that of the fine particles and vice-versa. As a matter of fact, the movement of both is favored at the same time. The logical conclusion, therefore, is that a decrease in viscosity rather than a change in specific gravity is concerned, though it is by no means unlikely that changes in specific gravity—whose effects are, however, relatively insignificant—may also occur.

In concluding the section on *Colpidium* it may be worth while to present in tabular form the behavior of this animal and of *Paramœcium* in a typical experiment where both organisms were obtained from the same culture and were subjected together to the effects of CO_2.

Experiments on Arbacia Eggs.

Certain experiments, as yet unpublished, which the author has made with a different end in view on the eggs of the sea-urchin, *Arbacia,* confirm to a certain extent the conclusions arrived at in the preceding sections. The experiments were primarily intended to throw light on the relative efficiency of buffered solutions of the same pH, but containing different amounts of CO_2 in producing internal changes in cells. For the purpose of detecting such changes, advantage was taken of the readiness with which centrifugal force brings about a stratification of the materials found in *Arbacia* eggs into four layers. This appearance was first described by Lyon ('07) and has since been used with good effect by Heilbrunn in following the changes in the viscosity of the protoplasm of these eggs at different stages of division, under the effects of anæsthetics, etc.

Table I.

Effect of Applying Centrifugal Force of 150 Times Gravity after Various Exposures to Carbon Dioxide.

Time of Exposure.	Paramœcium.	Colpidium.
1 minute.....	Slight evidences of liquefaction.	Slight evidences of liquefaction.
5 minutes.....	Liquefaction more distinct; body somewhat shrunken.	Liquefaction more distinct.
10 minutes.....	Practically all individuals show complete separation of food vacuoles.	Liquefaction very clear.
15 minutes.....	About the same; body beginning to swell.	Separation of food vacuoles almost complete.
20 minutes.....	Same; body more swollen.	Same; body somewhat swollen.
25 minutes.....	A few show failure of food vacuoles to separate.	Same as 20 minutes.
30 minutes.....	Same as 25 minutes.	Same as 20 minutes.
35 minutes.....	Same as 25 minutes.	A few show incomplete separation.
40 minutes.....	Many show no separation.	Less complete separation.
50 minutes.....	Most individuals show no separation.	More show absence of separation.
50 minutes.....	Absence of separation in practically all; recovery almost 100 per cent.	Separation absent in most individuals; recovery almost 100 per cent.
56 minutes.....	Same as 50 minutes; recovery about 75 per cent.	Same.
65 minutes.....	Same; recovery about 25 per cent.	Same.
75 minutes.....	All dead.	Same; many recoveries.

It is easy to demonstrate that carbon dioxide influences profoundly the consistency of the protoplasm of *Arbacia* eggs. In numerous experiments a five-minute exposure to sea water practically saturated with this gas was found to prevent stratification almost completely when the eggs were centrifuged for two minutes with a force estimated at 1,600 times that of gravity, while the controls in normal sea water showed the typical separation described by Lyon. That this result is due not to the hydrogen-ion concentration of the external medium as such, but rather to its carbon dioxide content (the carbonic acid probably penetrating the egg in an undissociated form or as CO_2 and subsequently dissociating within the cell), is shown by a comparison of the effects of

sea water to which CO_2 has been added with those of a similar solution in which the same pH has been produced by adding another acid—*e.g.*, HCl,—cautiously, drop by drop, and shaking vigorously after each addition to remove the CO_2 set free from the bicarbonates by the acid.

The results of one experiment of this sort may be given here. The exposures to carbon dioxide and to centrifugal force were those already mentioned. The numerical values of the pH of the solutions are only approximate, since the "salt errors" of the indicators used for determining them were disregarded, but as the error was in each case the same for the two solutions which were being compared, the general results obtained were not thereby affected.

TABLE II.

EFFECTS OF CENTRIFUGAL FORCE ON *Arbacia* EGGS EXPOSED FOR 5 MINUTES TO SEA WATER OF DIFFERENT pH VALUES.

pH (approximate)	pH regulated by adding CO_2.	pH regulated by adding HCl and then removing CO_2.
8.0	Stratification complete.	Stratification complete.
7.0	Stratification complete.	Stratification complete.
6.0	Stratification decidedly incomplete.	Stratification complete.
5.5	No stratification.	Stratification complete.

It appears from this experiment that the effects of carbon dioxide are primarily internal and depend on the absolute concentration of this substance rather than on the pH of the solution. These results are in agreement with those obtained by the author with other material and described in previous papers ('20-*a*, '20-*b*).

The solidifying effect of CO_2 is at least to a certain extent reversible. In several experiments eggs exposed to saturated sea water for 5 minutes were divided into two lots. The first lot was tested immediately and found to be solidified, giving no evidence of stratification after centrifugalization. The second lot was allowed to stand exposed to the air in a shallow dish for 30 minutes to permit the CO_2 to escape, or in some cases fresh sea water was also added. In either event centrifugal force produced normal stratification, showing that the eggs had again returned to the liquid

condition. Even the same eggs which had been centrifuged after exposure to CO_2 and had failed to stratify showed the normal behavior when the process was repeated after they had stood exposed to the air for 30 minutes.

It seemed desirable to determine whether in addition to this physical reversibility there was also complete physiological reversibility in the sense of permitting normal development to occur after solidification had been brought about. To test this point, a suspension of sperm was added to various lots of eggs after the CO_2 had been allowed to evaporate and the eggs had regained their fluidity. The results of these experiments were somewhat conflicting. In some cases no development occurred, in others there was cleavage as far as the 4- or the 8-cell stage, with perhaps irregular development beyond that point, while in still others normal development was obtained. The probable explanation of these differences was soon found to be that the exposure to CO_2 caused membrane formation (as had been noted by a number of previous observers) and subsequent development depended on whether or not conditions were favorable for complete artificial parthenogenesis. But whatever may have been the factors concerned in bringing about development, the mere fact that it occurred even in a portion of the cases where the eggs had undergone a very noticeable solidification is definite proof of the possibility of physiological reversibility.

It will be noticed that there have been described no liquefaction effects on *Arbacia* eggs. Whether or not such effects can be produced by lower concentrations or shorter exposures or both can be determined only when suitable material is again available. The experiments here described were made with another end in view before conditions in *Paramœcium* and *Colpidium* had been investigated, and the possibility of a preliminary liquefaction before solidification was not suspected. Though the experiments are, therefore, to this extent incomplete, their results, as far as they go, are in agreement with those obtained with the other kinds of material studied.

Experiments on Amœba.

The possibility of producing definite changes in the consistency of protoplasm by means of CO_2 suggested an interesting applica-

tion in the case of *Amœba*. It has repeatedly been urged by L. Loeb ('02), ('20), ('21), etc., that amœboid movement is due to changes in protoplasmic consistency. Hyman ('17) has also expressed the same view. The fact that carbon dioxide has been shown to cause such changes in the cases just described, and the additional consideration that it must be produced in an amœboid cell at a rate that varies greatly with circumstances, give some grounds for the hypothesis that the agent chiefly concerned in bringing about the protoplasmic changes underlying amœboid movement might be none other than this substance.

To determine to what extent the formation of pseudopodia can be influenced by carbon dioxide, an extremely fine capillary was made through which CO_2-saturated water could be discharged in small amounts. On several occasions quiescent amœbas (belonging to a species similar to, but probably not identical with, *A. Proteus*) were found, and on gently discharging a very small amount of the solution of CO_2 against the body of the animal pseudopodia were immediately put out in the direction of the capillary. On at least eight other occasions a small amount of the solution was discharged against the hindmost portion of a creeping amœba. In every case the same result was obtained. Pseudopodia were first put out at the point where the CO_2 came in contact with the body, the currents in the other pseudopodia being for a time reversed. Then as diffusion occurred and the full effects of the CO_2 became apparent all movements ceased for a few seconds, the animals remaining, as it were, congealed in the form in which they happened to be when overtaken by the effects of the dissolved gas. This paralysis then quickly passed away and movements were resumed in the original direction. To all appearances at least the animals had undergone a local liquefaction followed by a more general solidification. It follows from these rather crude experiments that if the external application of CO_2 can produce such results, it is not unlikely that internal effects of the same sort, resulting from varying rates of metabolism in different parts of the body, could conceivably give rise to amœboid movements of the usual type, though further work will be required to determine how far such an hypothesis explains the observed facts.

Experiments on Spirogyra.

It was thought desirable to supplement the experiments on animal cells with similar ones on some type of plant cell to determine how general the results already obtained might be. For this purpose *Spirogyra* was chosen, as it had already been successfully employed in a similar fashion by Szücs in his work with aluminum salts, and preliminary experiments showed that CO_2 produces marked differences in the ease with which the chloroplasts can be displaced by centrifugal force.

The first lot of *Spirogyra* studied, which was obtained early in the winter, showed clearly both liquefaction and solidification effects just as the Protozoa had done. Unfortunately, before all of the details of these processes could be studied, the material was exhausted and no more could be obtained until the following spring. This latter material, while giving the liquefaction effect with the greatest clearness, for some reason failed to show solidification except such as was obviously associated with irreversible death changes. The experiments, therefore, leave something to be desired in the way of completeness, but since the results, as far as they go, especially those obtained with the earlier material, are in good agreement with those already described, a few of them may be mentioned briefly.

In one experiment with the winter material a number of the filaments were placed for 2 minutes in distilled water saturated with CO_2. When centrifuged for 2 minutes at the same rate as that employed with the Protozoa, and then examined with the microscope, the chloroplasts in practically every cell were found to be aggregated in dense masses at the sides or ends of the cells, according to the position of the filament during centrifugalization. Controls in ordinary distilled water showed a much less complete separation. On the other hand, in filaments exposed for 10 minutes there was practically no movement of the chloroplasts at all, their spiral form being maintained almost as well as in cells which had been fixed for a few minutes in boiling water before centrifuging.

In another experiment filaments of *Spirogyra* were exposed to a saturated solution of carbon dioxide for 2, 4, 8 and 16 minutes,

respectively. The four lots, together with a control, were then centrifuged as before, with the following results: In the control some cells showed a separation of the chloroplasts, while others did not; in those exposed for 2 minutes practically all showed complete separation; in those exposed for 4 and for 8 minutes there was somewhat less separation (the amount being approximately the same as in the control), while with the 16-minute exposure there was very little separation and the filaments showed a certain apparent brittleness, being to some extent broken into pieces during their transfer to a watch glass for examination.

As to the reversibility of these effects on *Spirogyra,* it may be said that observations on this point are much more difficult to make than in the case of the motile Protozoa, and in the instances mentioned satisfactory determinations were not made. It has since been found with the more abundant material recently available that the filaments in which the exposure has been sufficient to produce decided liquefaction are not at all injured by the process, continuing to grow after the treatment in a normal manner. But whether or not recovery is possible after solidification can be determined only when *Spirogyra* which shows this behavior as clearly as did the first lot studied is again available in considerable quantities.

Discussion of Results.

It appears from the results obtained on four animal cells and on one plant cell that carbon dioxide can bring about changes in protoplasmic viscosity of two sorts. Clear evidence of liquefaction is obtained in *Paramœcium, Colpidium* and *Spirogyra.* Observations on this point are not available for *Arbacia* eggs, and in the case of *Amœba,* while the results are apparently in agreement with those already mentioned, they are based on mere appearances, and are therefore perhaps not so certain as the others. In every case where liquefaction was shown to occur this change could be shown to be reversible.

As to solidification, the clearest case is furnished by *Paramœcium,* where the process and its complete reversibility up to a certain point can easily be demonstrated. Conditions in *Colpidium* are almost as favorable, except that on account of the greater diffi-

culty in bringing about separation of the food vacuoles in the controls, the increasing viscosity can not be followed as far as in *Paramœcium*. In *Arbacia* eggs it is easy to produce solidification, which in the cases here described was in every case reversible, at least in the physical sense. On account of the complicating effects of artificial parthenogenesis the physiological reversibility is more difficult to demonstrate, but in a number of instances it was shown in an entirely satisfactory manner. In one lot of *Spirogyra* solidification was obtained, but could not be produced in several other lots subsequently studied, and until favorable material is again available it will be impossible to make any definite statements about the reversibility of the process. Finally, in *Amœba*, all of the appearances, at least, of solidification can be produced, though in this animal it is impossible to test the matter by the method of centrifugalization.

Considering in its entirety the evidence which has been given, there seems to be no doubt that carbon dioxide is an efficient agent for changing the consistency of protoplasm reversibly in either direction, the exact effects produced depending on the concentration of the gas, the material experimented upon, the time of exposure and the presence or absence of other dissolved substances in the medium. Since CO_2 has these properties, and since it is so universally present in cells and is produced at different rates at different times, it seems not unlikely that it may prove to be an important factor in bringing about certain of the changes in viscosity that accompany various physiological activities of cells. In the case of dividing eggs the temptation is strong to attempt to correlate the striking variations in the rate of CO_2 production observed by Lyon ('04) with the equally striking changes in protoplasmic viscosity described by Heilbrunn ('20), Chambers ('17) and others. The chief difficulty in this case would appear to be that since carbon dioxide can have either a liquefying or a solidifying effect, the exact nature of the changes produced by it would be difficult to predict in advance.

In the case of amœboid movement also the hypothesis that carbon dioxide may be at least an important agent is an attractive one. As a matter of fact, L. Loeb ('20) and Hyman ('17) have both expressed the idea that some substance is produced as the result of

metabolism which causes the necessary changes in protoplasmic consistency. L. Loeb ('20) has further suggested that a change in hydrogen-ion concentration is also concerned. It will be noted that carbon dioxide satisfies both of these requirements. In addition, the evidence presented in the present paper shows that it is capable of producing exactly the sort of changes in protoplasmic consistency demanded by such a theory.

It would be easy to engage in further speculations about the probable rôle of CO_2 in other physiological processes, but such speculations would not be warranted by the present state of our knowledge. It is suggested, however, on account of its universal occurrence in cells, as well as on account of its interesting and in some respects unique physiological peculiarities, that carbon dioxide should at least receive serious consideration in connection with many of the as yet unsolved physiological problems which involve changes in protoplasmic consistency.

Summary.

1. A short exposure to carbon dioxide of various cells causes a decrease, and a longer exposure an increase in the viscosity of the protoplasm.

2. Both of these effects are reversible, though the second one tends to pass into an irreversible coagulation if the exposure is sufficiently long continued.

3. The local application of carbon dioxide to *Amœba* can influence in a striking way the formation of pseudopodia.

4. It is suggested that carbon dioxide may be an important factor in producing many of the natural changes in protoplasmic consistency which have hitherto been unexplained.

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